

Using RAPDs to Detect QTLs in an Interspecific F_2 Hybrid of *Eucalyptus*

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Abstract

The inheritance and linkage relationships of Random Amplified Polymorphic DNA (RAPD) markers were examined in seedlings of an interspecific hybrid between *Eucalyptus gunnii* and *E. globulus* ssp. *globulus*. One hundred and ten RAPD markers were scored from 71 F_2 , 11 F_1 and 10 backcrosses to *E. gunnii*. Nineteen percent of the RAPD markers showed disturbed segregation in the F_2 , with most having a deficiency of *E. globulus* bands (13 out of 18). The F_2 had high levels of morphological abnormalities and poor growth compared to parental controls, F_1 and outcrossed F_2 progenies. Linkage analysis revealed fifteen linkage groups of three or more markers, 8 pairs and 13 unlinked markers ($LOD < 3.0$). Markers with disturbed segregation were clustered together. One normally segregating marker (U63-695) was significantly associated with abnormal leaf morphology, branching and frost tolerance. A significant proportion (ANOVA $R^2=18.5\%$) of the variation in frost tolerance in the F_2 was explained by this marker. However, abnormal leaf morphology and frost tolerance were not correlated. While interspecific hybridisation may be a powerful means of generating large amounts of genetic variation, the detection and exploitation of QTLs may be complicated by large and deleterious pleiotropic gene effects and biased by distorted segregation.

Introduction

Interspecific hybridisation is of interest in eucalypt breeding. Most attention has focused on the exploitation of F_1 hybrids. In many cases traits of interest are intermediate in F_1 hybrids and gains in one trait may only be made by compromising other traits of interests (Tibbits *et al.*, 1991). To provide suitable character combinations it may therefore be necessary to breed beyond the F_1 generation. However, in the case of forest tree species, there is little information on the extent to which interspecific hybrid incompatibility occurs or the manner in which traits are inherited in advanced generations. Hybridisation of *Eucalyptus globulus* ssp. *globulus* Labill. and *E. gunnii* Hook. f is of particular interest as this would allow the combination of genes of one of the faster growing, high pulp yielding species with genes of one of the most freezing-resistant species of *Eucalyptus*. The F_1 between the two species has intermediate frost tolerance, growth rate and morphology (Tibbits *et al.*, 1991). This intermediacy means that commercially the F_1 is unlikely to be competitive with more frost resistant species such as *E. nitens*. However, advanced generation breeding may allow the recombination of these traits (i.e. high frost tolerance, growth rate and pulp yield).

In the present study we investigated the inheritance and association of several quantitative characters (frost tolerance, growth rate, branching pattern, leaf shape, anthocyanin pigmentation) and molecular markers in F_1 , F_2 and backcross seedlings of a cross between *E. gunnii* and *E. globulus*. The objective was to develop a linkage map for RAPD markers and use this map to detect

quantitative trait loci (QTLs), showing the potential for marker aided selection.

Material and methods

A F_1 progeny between *E. gunnii* (GUN15) and *E. globulus* (GLOB23) was selfed upon flowering (F_2) and backcrossed (BC) to the original female parent (*E. gunnii*). The progenies were grown in a greenhouse. The cotyledon color was scored using a scale of 0 (none) to 4 (intense pigmentation) on the undersurface of the cotyledons. At 6.5 months of age a number of measures were taken: the total number of leaf pairs expanded; lamina width to length ratio and the relative position of the widest point of the lamina (using leaves excised from the tenth leaf pair, cotyledon=0); the number of laterals on the main stem divided by twice the number of nodes present (branch frequency); the relative length of the longest lateral compared to total plant height (branch length); seedlings exhibiting an abnormal leaf phenotype associated with chlorosis and distortion of the leaf margin were recorded. Frost tolerance was tested using the electroconductivity method (Tibbits *et al.*, 1991). Plants were hardened using a 16 hour night period (2 to 4°C) in a cold room and an 8 hour day period (22°C) for 22 days. Frost tolerance was measured at a single frost temperature (-4°C) that maximally differentiated *E. globulus* and *E. gunnii* when plants were 7.5 months old.

Total DNA was isolated from young leaf tissue using modifications of the CTAB method (Doyle and Doyle, 1990). The DNA content of the samples were quantified then assayed for the presence or absence of Random

Table 1. RAPD markers, their linkage group (? = unlinked) and level of significance of association with qualitative and quantitative traits measured from the *E. gunnii* x *E. globulus* F₂. Only associations significant at the 0.01 level are shown. Associations remaining significant after applications of the Bonferroni adjustment for multiple comparisons are shown in bold. Markers U63-695 and U280-1000 were linked together.

Trait	Marker and linkage group											
	U12-1300 3	U213-410 3	U16-730 5	U203-760 5	U2-1200 8	U230-800 12	U63-695 a	U280-1000 a	U2-1300 ?	U228-770 ?	U43-1300 ?	U54-980 ?
Lamina width/length	.0081										.0041	
Position of the widest point of the lamina			.0019	.0019								
Leaf pairs expanded		.0054						.0085				.0092
Abnormal leaf morphology		.0017					1 x 10 ⁻⁷	.0011				.0082
Branching frequency						.0026	.00008					.0016
Branch length					.0062		.0011					
Frost tolerance							.0013					
Cotyledon color									.0082	1 x 10 ⁻⁷		

Amplified Polymorphic DNA (RAPD) markers (Williams *et al.*, 1990). Primers were obtained from Operon Technologies Inc and the University of British Columbia. Amplification conditions were as in Williams *et al.*, (1990) except that 150mg/mL of BSA was added to each reaction and 10 ng of DNA was used in a total reaction volume of 15uL. Consistency of interpretation was established by repeated blind scoring and by running part of the progeny in a separate RAPD batch.

Goodness of fit to expected genetic ratio (3:1) was tested using C². Linkage analysis was performed using JoinMap (Stam, 1993) combining F₂ and backcross individuals at LOD=3.0. Association between RAPD bands and Quantitative trait loci (QTL) in the F₂ was tested using one way ANOVA (GLM; SAS, 1990) using markers as the treatment. To account for type I errors through multiple comparison, the sequential Bonferroni correction was applied at the individual trait level.

Results

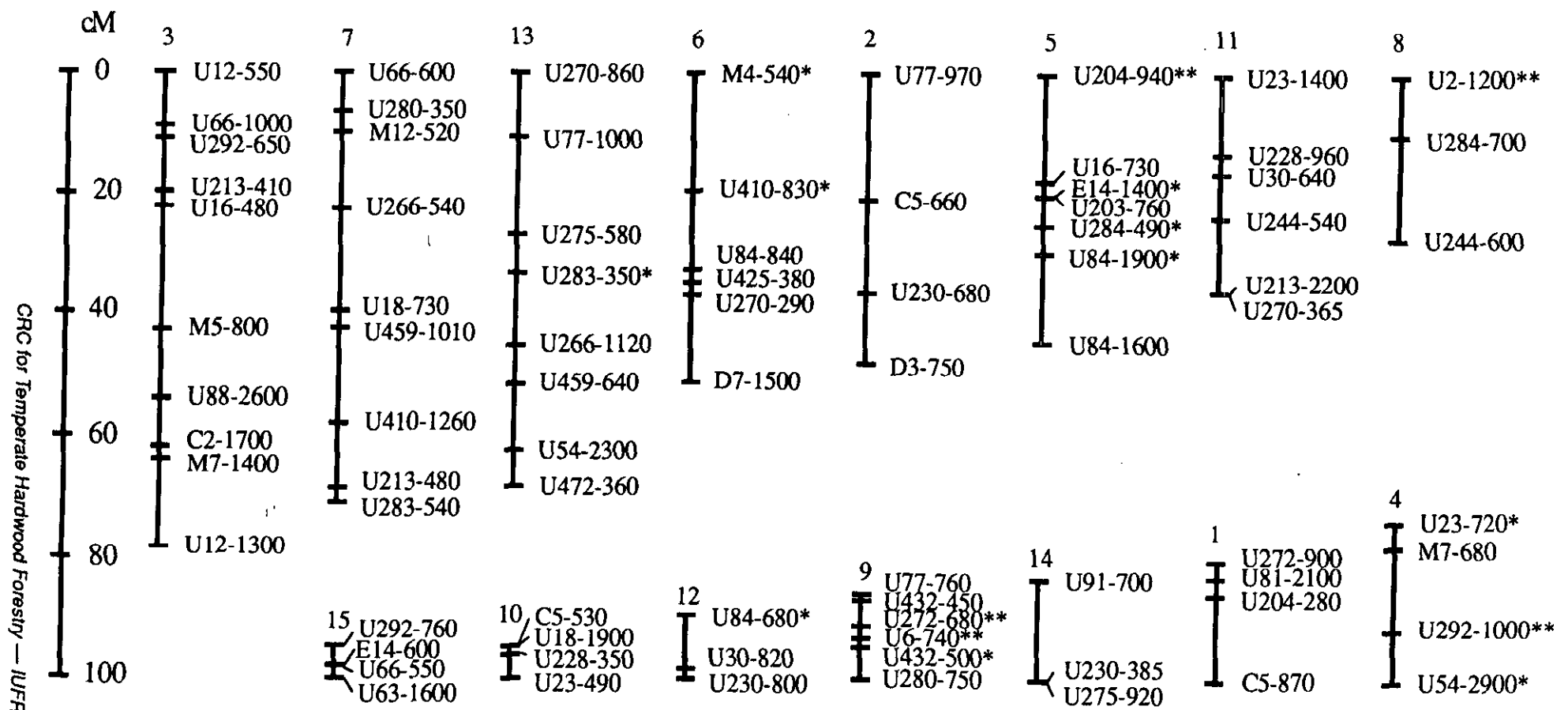
One hundred and ten segregating RAPD markers were scored among the 72 F₂, 11 F₁ and 10 BC plants. Twenty one of these 110 markers (19.1%) gave significant deviation from 3:1 expected segregation in the F₂ (9 at P<0.01 and 12 at P<0.05). Thirteen markers showed a deficiency in *E. globulus* bands, five a deficiency in *E. gunnii* bands, and for three markers the genotype of the parents was unknown. The number of markers showing deficiency in *E. globulus* and *E. gunnii*

genetic material was significantly different from a 1:1 distribution (X²=4.98*). The F₂ had high levels of morphological abnormalities and poor growth compared to parental controls, F₁ and BC plants as well as crosses between unrelated F₁ hybrids (outcrossed F₂).

Fifteen linkage groups of three or more markers were formed when using together the 72 F₂ and 10 BC plants (LOD>3.0, Fig. 1). Eight linkage groups of two markers were formed and 13 markers remained unlinked after the linkage analysis using JoinMap. Four linkage groups (#4, #5, #6 and #9) had two or more markers with disturbed segregation. Linkage group 4 had three out of four markers with disturbed segregation, all three with a deficiency in *E. globulus* bands. Linkage group 5 had four markers with disturbed segregation, all four with a deficiency in *E. globulus* bands. Linkage group 6 had two markers with disturbed segregation, one with a deficiency in *E. globulus* bands, the other with a deficiency in *E. gunnii* bands. In linkage group 9, two of these markers had a deficiency of *E. gunnii* bands, a third marker with disturbed segregation was also linked to these, but in this case the genotype of the parents for this band was unknown, therefore the direction of the disturbed segregation was unknown.

Of the 8 morphological characters screened by ANOVA for association with the 110 RAPD markers, 19 associations were observed at the 0.01 level (Table 1). Three associations involving two markers remained significant when the stringent Bonferroni adjustment was

Figure 1. RAPD linkage groups (LOD = 3.0) detected in the F_2 and backcross of an interspecific cross between *E. gunnii* and *E. globulus*. Map-distance is given in centiMorgans. The name of the markers includes the primer and the size (in base pairs) of the amplified band. Markers with disturbed segregation are indicated with asterisk corresponding to the level of significance of the segregation distortion.



used to account for multiple comparisons.

Segregation for abnormal leaf morphology was observed in the F_2 . Fifty four percent of the F_2 had leaves with irregular margins. This trait was not segregating in the F_1 , in open-pollinated progenies of GLOB23, outcrosses of GUN15 to unrelated *E. gunnii* parents or backcrosses to unrelated *E. globulus* parents. Segregation was observed in open-pollinated GUN15 (which may include selfs) and the backcross to GUN15. This abnormal leaf morphology was associated with poor plant vigour (Spearman r with plant height = -0.36, $P=0.002$ and leaf pairs expanded $r=-0.54$, $P<0.001$) and lack of apical dominance (branching frequency $r=0.50$, $P<0.001$ and branch length $r=0.39$, $P=0.001$). ANOVA indicated that one normally segregating RAPD marker (U63-695) was associated ($P<0.001$) with variation in this phenotype (Table 1). U63-695 also explained a significant proportion of the variation for branching frequency ($R^2=21\%$, $P<0.001$), branch length ($R^2=19\%$, $P<0.001$) and frost tolerance ($R^2=19\%$, $P<0.005$). There was no significant correlation between frost tolerance and abnormal leaf morphology (Spearman $r=0.23$, $P=0.08$). Cotyledon color was significantly associated with two markers U228-770 and U2-1300 and together these markers accounted for 52% of the variation in cotyledon color.

Discussion

Fifteen linkage groups were detected using the 110 RAPD markers. Most *Eucalyptus* species have a haploid number of eleven chromosomes (Eldridge *et al.*, 1993). The fact that we found more linkage groups than there are chromosomes indicates that our linkage map is not saturated. Nevertheless, we have been successful in identifying associations between markers and QTL's. Abnormal leaf morphology is associated with the same marker (U63-695) as the QTLs for both frost tolerance and branching. In the case of frost tolerance there may be an independent QTL in the region of U63-695, since the association between abnormal leaf development and our measure of frost tolerance is not significant. In contrast, the association of U63-695 with branching frequency and branch length appears to be a pleiotropic effect of abnormal leaf morphology. Intercorrelated characters and pleiotropic effects are potentially a major problem in QTL analysis which has received little attention.

Two separate types of disturbing phenomena were evident in the interspecific F_2 , distorted segregation of RAPD markers and abnormal morphology. Abnormal leaf morphology did not account for the presence of distorted segregation since the markers associated with abnormal leaf morphology had normal segregation. The high frequency of markers with distorted segregation, the clustering of the markers and the directionality of the allele loss could arise from karyotype differences between the two species causing loss of chromosome pieces and differential loss of alleles. The high level of

morphological abnormalities and the poor performance of our *E. gunnii* x *E. globulus* F_2 may be largely due to exposure of a major deleterious gene through selfing as opposed to interspecific hybridisation *per se*, since high frequencies of abnormalities were not observed when the F_1 hybrid female was crossed to an unrelated F_1 (unpubl. data). The allele(s) contributing to this deleterious effect appear to have been derived from the *E. gunnii* female and to be located in the vicinity of the RAPD marker U63-695. In *E. grandis* x *E. urophylla* F_1 crosses (Grattapaglia and Sederoff 1993) no disturbed segregation of RAPD markers was detected, probably because there was no recombination between parental genomes.

In conclusion, RAPD markers have been used successfully to construct a linkage map for an interspecific hybrid of *Eucalyptus* and the map used to locate QTLs. In our case, segregation for abnormal leaf morphology may have masked other QTLs and exploration of other *E. gunnii* x *E. globulus* hybrid combinations is warranted. While interspecific hybridisation may be a powerful means of generating large amounts of genetic variation, the detection and exploitation of QTLs may be complicated by large deleterious pleiotropic gene effects and biased by disturbed segregation.

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